

Preparation of Water-Soluble Metal Complexes of Rutin and Related Flavonols*

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Because of the need for means of solubilizing rutin, a number of flavonoid metal complexes were prepared. Several of these, made from rutin in combination with iron compounds, appear to combine with rutin in a stoichiometric relationship. Particularly effective in solubilization of rutin is colloidal saccharated iron oxide. Solutions of rutin in saccharated iron oxide may be varied over an appreciable concentration range; they are stable, pleasant to taste, and should be tolerated by intravenous injection, since solutions of saccharated iron oxide alone are well tolerated.

THE EFFECTIVENESS of rutin in restoring increased capillary fragility and permeability in man to normal was established in 1943 by Griffith, Lindauer, and Couch and disclosed in a report to the Medical Society of the State of Pennsylvania (7), followed a year later by the publication of results (5).

Subsequently, rutin proved so effective that by 1946 over 1,000 case histories were available for evaluation. By using actuarial tables, it can be shown from these data (4) that when hypertension is complicated by capillary fault, patients not treated with rutin have a mortality rate 8.5 times normal, while patients treated with rutin have a mortality rate only 1.5 times normal. Treatment with rutin reduces the mortality rate due to apoplexy by about 90 per cent and that due to coronary occlusion by about 50 per cent.

The chemistry, pharmacology, and clinical applications of rutin have been summarized recently by Couch, *et al.* (3).

Usually rutin is administered to patients in the form of 20- or 50-mg. tablets, and the daily dose is 60 mg. (and upward). Because of its limited solubility (about 0.1 Gm. per liter in cold water) rutin has, of necessity, been given orally. Taken in this fashion it may require some time for the drug to be assimilated and become effective in restoration of capillaries to a state of reduced fragility. At any rate, it is not known how quickly orally administered rutin is assimilated, as an interval of three to four weeks must elapse before a repeat of the Gothlin test (for capillary fragility) is made (6). The need for rutin in soluble form is apparent from the number of requests received from industry and from physicians, es-

pecially physicians attending patients who have had gastrointestinal hemorrhage, for whom oral therapy may be considered inadvisable.

To date, the only published report of a water-soluble form of rutin found is that of Plungian (9) on the solubilization of rutin with methyl glucamine. In view of the need for water-soluble rutin for oral and injection purposes, further studies were initiated. The metal compounds given particular attention as possible solubilizing agents were those of iron, copper, manganese, and cobalt because of their added nutritional significance. *Nutrition Reviews* suggests situations where intravenous iron should be helpful (8).

For the most part, selection of various iron compounds for study was based upon therapeutic usage as indicated by inclusion in the United States Pharmacopeia or the National Formulary. Literature articles on the treatment of hypochromic anemia with ferrous gluconate administered either intramuscularly or orally (10) and with saccharated iron oxide intravenously administered (1, 2, 12) led to particular interest in these forms of iron as solubilizing agents for rutin.

EXPERIMENTAL

Solubilization of Rutin with Iron Compounds

A number of iron salts were tested with respect to their ability to solubilize or complex with rutin. The general procedure was as follows:

Purified rutin, $C_{27}H_{30}O_{16} \cdot 3H_2O$, N. F. IX (quercetin content, 0.8%), 0.70 Gm., was dissolved in approximately 150 ml. of boiling distilled water (almost a saturated solution). To boiling solutions of this strength were added varying amounts, from 0.25 to 4.00 moles, of iron compounds to be tested. After boiling about one minute, the solutions were filtered, cooled, each made to a volume of 150 ml., and the pH was determined. They were allowed to stand for twenty-four hours in stoppered bottles. At the end of this period, the solutions were filtered, and the residue was washed with portions of cold distilled water (total 5 ml.). The residue (unreacted rutin) was dried at 110° for several hours, then allowed to equilibrate with air to constant weight in

* Received October 24, 1951, from the Eastern Regional Research Laboratory, Philadelphia 18, Pa., one of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

†The authors are indebted to Mr. Arthur Turner, Jr., for spectrophotometric analyses and Dr. James Sprague of Sharp and Dohme, Inc., for saccharated iron oxide.
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TABLE I.—EFFECT OF IRON SALT CONCENTRATION UPON RUTIN SOLUBILITY

Moles of Iron Compound per Mole of Rutin ^a	Iron Compound			
	$\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}^b$	$\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$	$\text{Fe}[\text{CH}_2\text{OH}(\text{CHOH})_4\text{COO}]_3 \cdot 6\text{H}_2\text{O}$	$\text{Fe}_2(\text{P}_2\text{O}_7)_3 \cdot 9\text{H}_2\text{O}$
	Weight of Unreacted Rutin, Gm.			
0.25	0.63	0.50	0.54	0.59
0.50	0.65	0.35	0.02	0.54
0.75	0.60	0.20	0.02	0.49
1.00	0.01	0.04	Trace	0.49
2.00	None	None	Trace	0.37
4.00	None	None	None	0.24
	pH of Filtrate			
0.25	3.2	2.7	3.7	5.6
0.50	3.2	2.4	3.7	5.2
0.75	3.2	2.3	3.9	5.2
1.00	3.3	2.1	3.9	5.2
2.00	3.3	1.9	4.2	5.2
4.00	3.4	1.6	4.2	4.9
	Weight of Iron Compound, Gm.			
0.25	0.10	0.07	0.13	0.24
0.50	0.20	0.14	0.25	0.48
0.75	0.31	0.21	0.38	0.72
1.00	0.41	0.28	0.51	0.96
2.00	0.82	0.56	1.02	1.92
4.00	1.64	1.12	2.04	3.84

^a Rutin (0.700 Gm.) dissolved in 150 ml. of boiling water.^b Ferric ammonium sulfate $[\text{Fe}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}]$, 0.51 Gm. (0.50 mole), gave no unreacted rutin; pH of filtrate, 2.3.

order to compare its weight with that of original rutin used. Table I indicates that rutin was successfully solubilized by ferrous ammonium sulfate, ferric chloride, and ferrous gluconate, and only partially by ferric pyrophosphate. In addition to these, this same quantity of rutin was completely solubilized by 0.51 Gm. (0.50 mole) of ferric ammonium sulfate, $\text{Fe}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$, giving a solution of pH 2.3; and by 0.25 Gm. (1 mole) of ferrous lactate, $\text{Fe}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$, N. F. V, giving a solution of pH 3.3.

It appears from these data that ferrous ammonium sulfate, ferric chloride and ferrous gluconate (and possibly ferric ammonium sulfate and ferrous lactate) bear a stoichiometric relationship to the rutin used. It would seem that rutin reacts in an equimolecular ratio with ferrous ammonium sulfate and ferric chloride, whereas 2 moles react with each mole of ferrous gluconate.

Several of these solutions (ones containing ferrous gluconate and ferric chloride) were examined spectrophotometrically. The maxima in the ultraviolet range were not markedly different from those of rutin or the iron compound involved, although slight spectral changes were observed in the visible range.

From the amounts of unreacted rutin obtained with ferric pyrophosphate (Table I), it appears that

no complex is formed, although varying amounts of this compound do seem to have some solubilizing effect, influenced by the iron salt concentration.

Only when ferrous sulfate was combined as ferrous ammonium sulfate did it solubilize rutin. Ferrous sulfate alone, tried at different concentrations, failed to solubilize the flavonol. Data illustrating that combinations of ferrous sulfate and ammonium sulfate fail to solubilize rutin are presented in Table II. Other iron salts which failed to solubilize rutin when tried at different concentrations were: ferric sulfate, ferric ammonium citrate, ferric glycerophosphate, ferrous phosphate, and ferrous chloride. In the case of ferric ammonium citrate, different amounts of citric acid were added to determine whether change in pH might have any effect; there was none.

At about the time the work described above was completed, the previously mentioned article by Brown, *et al.* (2), on saccharated iron oxide came to our attention. Experiments have since been directed to the more practical aspects, such as methods of preparation of solutions containing possible therapeutic doses of rutin with ferrous gluconate and saccharated iron oxide.

Rutin-Ferrous Gluconate Preparations.—A stable solution of rutin and ferrous gluconate containing approximately 0.3 Gm. (N. F. IX usual dose) of

TABLE II.—EFFECT OF VARIOUS COMBINATIONS OF FERROUS SULFATE AND AMMONIUM SULFATE ON RUTIN SOLUBILITY 0.700 GM. OF RUTIN IN 150 ML. OF WATER

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$		$(\text{NH}_4)_2\text{SO}_4$		pH of Filtrate	Weight of Unreacted Rutin, Gm.
Mole/Mole Rutin	Weight, Gm.	Mole/Mole Rutin	Weight, Gm.		
1	0.31	0.1	0.014	3.4	0.65
1	0.31	0.2	0.028	3.4	0.63
1	0.31	0.4	0.056	3.3	0.65
1	0.31	0.8	0.112	3.3	0.62
1	0.31	1.0	0.140	3.5	0.56
1	0.31	2.0	0.280	3.4	0.65
2	0.62	2.0	0.280	3.4	0.65

ferrous gluconate and 20 mg. (N. F. IX usual dose) of rutin per 5 ml. was prepared by dissolving 0.11 Gm. of rutin in 20 ml. of boiling water (saturated solution of rutin) and adding 1.50 Gm. of ferrous gluconate, N. F. IX (final concentration about 6% ferrous gluconate). The resulting dark-red solution was filtered while hot, cooled, and made to a volume of 25 ml. This solution had a pH of 4.1, was stable, and did not become moldy on storage in an unsterilized bottle for at least five weeks.

Similar solutions containing up to about 2.5% of dissolved rutin were prepared by this procedure; a 6% solution of ferrous gluconate as solvent was used. On standing for some time, a small amount of dark-green water-insoluble material appears, probably an oxidation product (11).

For convenience, rutin and ferrous gluconate may be mixed in powder form. For example, when the minimum amount of iron is desired, 0.55 Gm. of rutin can be mixed with 0.20 Gm. of ferrous gluconate. This mixture can then be dissolved in 100 ml. (measured cold) of boiling water. The dark-red boiling solution is filtered, cooled, and diluted to volume (100 ml.). The pH of the filtrate is 4.2; each 5 ml. of solution contains 28 mg. of rutin and 1.16 mg. of iron.

Rutin-Saccharated Iron Oxide Preparation.—A stable, aqueous colloidal rutin-saccharated ferric oxide preparation was made by dissolving 50.0 Gm. of saccharated ferric oxide, N. F. VII, (contained 3.1% iron) in 70 ml. of distilled water (pH of solution, 9.7). The volume became approximately 100 ml. when the solid was completely dissolved. When the solution was heated to boiling, different amounts of rutin, up to 6.0 Gm., could be dissolved. The solutions were filtered, cooled, and made to a volume of 100 ml., and their pH was measured. (Solutions so prepared may be diluted to any strength with water without precipitation of rutin.) Five milliliters (about a teaspoonful, a convenient amount for injection) of these solutions contained the average N. F. dose of 2 Gm. of iron compound and up to 550 mg. of rutin (N. F. average dose, 20 mg.).

Solutions prepared as described above are stable for at least six months when stored in stoppered glass bottles. Such solutions prepared with more than 1.5 Gm. of rutin occasionally set to a thixotropic gel.

The pH range of these solutions was from 6.8 (for 6.0 Gm. of rutin per 100 ml.) to 9.3 (for 0.40 Gm. of rutin per 100 ml.; this was a solution each 5 ml. of which contained 20 mg., the average N. F. dose). The pH of solutions containing 1.5, 2.0, and 3.0 Gm. of rutin was 8.0, 7.7, and 7.3, respectively. These preparations are sweet and pleasant to taste and do not have the usual astringent characteristic of iron compounds.

Solubilization of Rutin with Other Metal Salts

Copper.—A stable, aqueous, rutin cuprous chloride preparation was made by dissolving 0.70 Gm. of rutin in 150 ml. of boiling distilled water and adding 0.20 Gm. of the copper salt. The resulting deep orange-yellow solution containing the glycoside and metal salt in a mole ratio of about 1:2 had a pH of 4.0. (Unreacted rutin, 0.05 Gm. after twenty-four hours, was obtained from this solution, and the same amount of unreacted rutin was obtained from a 1:3 ratio.) One mole of cuprous chloride did not com-

plex and 1 or 2 moles of cupric sulfate did not complex with 1 mole of rutin when treated under similar conditions.

Cobalt and Manganese.—Rutin in boiling solutions under conditions similar to those described above for copper, failed to complex with 1 and 2 mole ratios of cobaltous chloride, manganous chloride, or manganous sulfate.

Solubilization of Other Flavonols with Iron Compounds

Ferrous Gluconate Solutions as Solvents.—Quercetin ($C_{15}H_{10}O_7$) in any proportion failed to react with a 6% solution of ferrous gluconate.

Quercitrin ($C_{21}H_{22}O_{13} \cdot 2H_2O$), however, was solubilized by a boiling 6% solution of ferrous gluconate; one containing 1.50 Gm. of iron salt in 25 ml. of water dissolved up to 0.30 Gm. of quercitrin. The formation of considerable black solid (0.06 Gm.) on cooling made it appear impractical to proceed further with ferrous gluconate as a solubilizing agent for quercitrin.

TABLE III.—SOLUBILITY OF FLAVONOLS IN SOLUTIONS CONTAINING 50.0 GM. OF SACCHARATED IRON OXIDE PER 100 ML. OF WATER

Flavonol	Weight of Flavonol Soluble, Gm.	pH of Solution
Rutin	6.0	6.8
Quercetin	1.5	8.0
Quercitrin	3.0	7.5
Quercetagetin	2.0	7.7
Robinin	3.3	8.8

Saccharated Iron Oxide Solutions as Solvents.—Solutions of quercetin, quercitrin, quercetagetin ($C_{15}H_{10}O_7 \cdot 2H_2O$) and robinin ($C_{23}H_{24}O_{20} \cdot 8H_2O$) were prepared in a manner similar to that described for rutin; 50.0 Gm. of saccharated iron oxide per 100 ml. of distilled water was used. Table III summarizes the information obtained. The quantity of flavonol indicated in each case is near the maximum solubility for that flavonol. These solutions were all stable for at least six months when stored in glass-stoppered bottles.

SUMMARY

Numerous iron salts were tested to determine their ability to increase the solubility of rutin in water. Ferrous ammonium sulfate, ferric chloride, ferrous gluconate, ferric ammonium sulfate, and ferrous lactate solubilized rutin by reacting in a manner which appears to be stoichiometric. Also, rutin was solubilized by colloidal saccharated iron oxide.

Ferric sulfate, ferric ammonium citrate, ferric glycerophosphate, ferrous phosphate, and ferrous chloride failed to solubilize rutin. Cuprous chloride, but not cupric sulfate, cobaltous chloride, manganous chloride, and manganous sulfate, solubilized rutin.

Ferrous gluconate failed to solubilize quercetin and quercitrin.

In addition to rutin, colloidal saccharated iron oxide solubilized quercetin, quercitrin, quercetin, and robinin.

Of the various metal combinations found effective in solubilizing rutin, colloidal iron oxide appears to offer the most promise.

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